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Bacterial Etiology of Community Acquired Pneumonia and their Antimicrobial Susceptibility in Patients Admitted to Alshaab Teaching Hospital, Sudan.

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Abstract

Background: Community Acquired Pneumonia (CAP) is a frequently encountered lower respiratory tract parenchymal lung infection which continues to be a major health problem leading to significant morbidity and mortality worldwide.

Objective: This study was aimed to determine frequency and susceptibility pattern of bacterial etiology of community acquired pneumonia isolates from Sudanese patients.

Materials and methods: One hundred Sputum specimens were collected from patients with Community Acquired Pneumonia attended to chest unit in Alshaab Teaching Hospital during the period between Januarys to March 2017. The patients were grouped according their age as follows (year); 16 to 26, 27 to 37, 38 to 48, and 49 to 60.Antibiotic susceptibility test of isolated organisms were carry out by the Kirby-Baur disc diffusion method.

Results: Out of 100 patient enrolled in the study the etiology was identified in 42/100 (42%) with most frequent isolates *Klebsiella pneumoniae* 18/42 (42.8%) followed by: *Pseudomonas aeruginosa* 13/42 (30.9%), *Staphylococcus aureus* 10/42 (23.9%) and *Escherichia coli* 1/42 (2.4%). Only 3 (16.7%) organisms among all isolated *Klebsiella pneumoniae* were found resistant to all investigated antibiotic (third generation cephalosporin (3GC)) 6/10 (60%) from all isolated *Staphylococcus aureus* found resistant to methicillin (ME 10 µg) and Oxacillin (OX 1 µg). All isolated *Pseudomonas aeruginosa* were sensitive to Meropenem. The isolated *Escherichia coli* found only resistant to Ampicillin.

Conclusion: Prevalence of community-acquired pneumonia in adult Sudanese patients is relatively high with major causative agents *Klebsiella pneumoniae*.

Keywords: Community-acquired pneumonia (CAP), *Klebsiella pneumoniae*, Alcoholic abuse, Methicillin resistant *Staphylococcus aureus* (MRSA).

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Introduction

Community-acquired pneumonia (CAP) is a common lower respiratory tract infection and remains an important cause of morbidity and mortality worldwide [1,2]. Detailed information on the etiology of CAP is required for the formulation of treatment recommendations and the introduction of preventive measures [3]. Up to 36% of patients who have CAP require admission to the ICU [4]. The range of microorganisms reported as being responsible for community acquired pneumonia varies between studies and depends on the patient population studied, the extent of laboratory investigations, and the microbiological techniques employed in some Studies have identified cause of pneumonia were *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and other respiratory viruses as the main pathogens associated with CAP [5]. The distribution of etiological pathogens of CAP may vary by country. Most of the data on pathogen distribution of CAP have been reported from western countries, whilst only a few data are available in the Asian region [6]. The initial management of patients suspected of having community-acquired pneumonia is challenging because of the broad range of clinical presentations, potential life-threatening nature of the illness, and associated high costs of care. The initial testing strategies should accurately establish a diagnosis and prognosis in order to determine the optimal treatment strategy [7].

Method

Descriptive cross sectional Hospital base study was performed from December 2016 to February 2017. A total of 100 patients attended into chest unit in Alshaab Teaching Hospital with sign and symptoms of lower respiratory tract infection were enrolled in this study.

All sputum samples were collected from enrolled patient in sterile container with screw cap and transported to research laboratory according to Clinical Laboratory Standard Institute (CLSI).

Then inoculated under aseptic technique in 5% Blood agar, MacConkey agar and Chocolate agar prepared according to manufacturer's instruction, the inoculated culture media were incubated aerobically at 37°C overnight and culture of chocolate agar incubated at 37°C in candle jar overnight.

Smears from detected growth were prepared for Gram stain which was used as basic identification of possible causative agents, for Gram positive cooci catalase test was performed, for catalase positive organism DNAse test, coagulase test and Novobiocin sensitivity test was done to identify *Staphylococcus spp.* Gram negative identification was done by using motility test, oxidase test, Kligler iron agar, cirate utilization test, urease hydrolysis test, indole production test and methyl red test. Antibiotic susceptibility test of isolated organisms, the test was performed using Kirby-Bauer disc diffusion method as follows:

Preparation of inoculums

The inoculums were prepared by transfer 2-5 well isolated colony of same appearance with sterile wire lope to 2 ml of sterile physiological saline. The turbidity of suspension was adjusted to a 0.5 McFarland standard these suspensions were used within 15 min of preparation.

Seeding of plates

Sterile nontoxic cotton swab was dipped into the inoculums tube and then the swab was rotated against the side of the tube above the level of suspension to remove excess fluid.

The plate of Muller Hinton agar was inoculated by streaking the swab evenly over the surface of the medium in three directions the surface of agar was allowed 3-5 min to dry.

Antibiotic disc application

The selected antibiotics were applied on surface of agar by using sterile forceps which evenly distributed in the inoculated plate each disc was pressed down to ensure its contact with the agar.

Incubation

The inverted plates were incubated aerobically at 37°C for 18-24 h.

Reading of zones of inhibition

Following overnight incubation by using a ruler on the underside plates the diameter of each zone of inhibition was measured in millimetres.

Interpretation of result

The zone of each antibiotic was compared to their standard inhibition zone on the chart provided by manufacture the result were interpreted as sensitive (S), intermediate (I) or resistance (R).

Methicillin (10 µg), Oxacillin (1 µg) was used to detect community acquired methicillin resistance *Staphylococcus aureus* (MRSA). Susceptibility test for *Klebsiella pneumonia* was done by using standard disc diffusion (SDDF) as screaming method for identifying potential extended spectrum B-lactamase (ESBLs) against a group of third generation cephalosporin (3GC) include Ceftazidime (CAZ, 30 µg), Ceftriaxone (CRO, 30 µg), Cefepime (FEP, 30 µg), Cefotaxime (CTX, 30 µg). Standard double disk synergy test (DDST) was done to *Klebsiella pneumonia* which found resistant to the group of cephalosporin (3GC) antibiotic. Eight antibiotic discs were tested for *Pseudomonas aeruginosa*, include Meropenem (MEM, 10 µg), Cefepime (FEP, 30 µg), Ceftazidime (CAZ, 30 µg), Gentamicin (CN, 10 µg), Colistin (CT, 10 µg), piperacillin (PRL, 100 µg), Aztreonam (ATM, 30 µg), Imipenem (IPM, 10 µg). Ten commercially available antibiotic discs were used for *Escherichia coli* antibiotics were Ampicillin (10 µg), Amoxicillin (25 µg), Gentamycin (10 µg), Cefixime (5 µg), Amikacin (30 µg), Ceftazidime (30 µg), Ciprofloxacin (5 µg), Imipenem (10 µg), and Norfloxacin (10 µg), Trimethoprim/ Sulfamethoxazole (1.25 µg/23.75 µg).

Results

The results showed 42 (42%) were positive for bacterial growth. Out of investigated specimen CAP agents were identified as 18/42 (42.8) *Klebsiella pneumoniae* followed by 13/42 (30.9%) *Pseudomonas aeruginosa*, 10/42 (23.9%) *Staphylococcus aureus* and 1/24 (2.4%) *Escherichia coli*; CAP agents were more prevalent in males 23/42 (54.7%) than females 19/42 (45.3%) but this result was statistically insignificant (P-value=0.129) (Table 1).

Table 1. *Frequency of isolated bacteria according to gender.*

Isolated bacteria	Gender		Total
	Male	Female	
Klebsiella pneumonia	7 (16.6%)	11 (26.3%)	18 (42.8%)
Pseudomonas aeruginosa	8 (19%)	5 (11.9%)	13 (30.9%)
Escherichia coli	1 (2.4%)	0	1 (2.4%)
Staphylococcus aureus	7 (16.7%)	3 (7.2%)	10 (23.9%)
Total	23 (54.7%)	19 (45.3%)	42 (100%)

(P-value=0.129).

The result showed insignificant relationship between isolated bacteria and smoking (P-value=0.899). CAP agents were found

significantly more frequent among patients with alcohol abuse show in Table 2.

Table 2. *Relationships between isolated bacteria and alcoholism.*

Isolated bacteria	Alcoholism		Total
	Yes	No	
Klebsiella pneumonia	0	18	18
Pseudomonas aeruginosa	2	11	13
Escherichia coli	0	1	1
Staphylococcus aureus	3	7	10
Total	5	37	42
P-value=0.025			

The result of standard disc diffusion (SDDF) which used as screaming method for identifying potential extended spectrum B-lactamase (ESBLs) producer was done against a group of third generation cephalosporin (3GC) include Cefepime (CPM), Ceftriaxone (CL), Ceftazidime (CAZ) and Cefotaxime (CTX) the test result show that only 3 (16.7%) organism among all isolated *Klebsiella pneumoniae* were resistant to all investigated antibiotics while remain 15 (83.3%) were sensitive as show in Table 3.

Table 3. *Antimicrobial susceptibility of Klebsiella pneumoniae against third generation cephalosporin.*

Antibiotic discs	Inhibition zone		Total
	Sensitive	Resistant	
Ceftazidime	15	3	18
Cefepime	15	3	18
Ceftriaxone	15	3	18
Cefotaxime	15	3	18
Total	60	12	72

The Double Disc Synergy Test (DDST) was done to these 3 organism which showed resistant to the group of cephalosporin (3GC) antibiotics which indicated that these 3 organism could considered as ESBLs producers none of ESBLs harbouring isolate was susceptible to combination of amoxicillin and clavulanic acid. Antibiotic susceptibility of *pseudomonas aeruginosa* that includes eight antibiotics detected that all isolates were 100% sensitive to Meropenem follow 92.3% sensitive to fifth antibiotics they were Cefepim, Ceftazidime, Gentamicin, Aztreonam, Imipenem, followed by 84.6% sensitive for Colistin, the less effective antibiotics is Piperacillin with 53.8% sensitivity as show in Table 4.

Table 4. *Antimicrobial susceptibility test of Pseudomonas aeruginosa against some antibiotic.*

Antibiotic discs	Inhibition zone			Total
	Sensitive	Resistant	Intermediate	
Meropenem	13	0	0	13
	100.0%	0.0%	0.0%	100.0%
Cefepim	12	1	0	13
	92.3%	7.7%	0.0%	100.0%
Ceftazidime	12	1	0	13
	92.3%	7.7%	0.0%	100.0%
Gentamicin	12	1	0	13
	92.3%	7.7%	0.0%	100.0%
Colistin	11	2	0	13
	84.6%	15.4%	0.0%	100.0%
Piperacillin	7	6	0	13
	53.8%	46.2%	0.0%	100.0%
Aztreonam	12	1	0	13
	92.3%	7.7%	0.0%	100.0%
Imipenem	12	0	1	13
	92.3%	0.0%	7.7%	100.0%
Total	91	12	1	104
	87.5%	11.5%	1.0%	100.0%

Antimicrobial susceptibility test for Staphylococcus aureus

Ten isolated *Staphylococcus aureus* were tested using 14 antibiotics which all isolates show 90% sensitive to Ceftriaxone (CRO, 30 µg) and Amikacin (AK, 30 µg), followed by 80% sensitivity to Vancomycin (VA, 30 µg), Clindamycin (DA, 2 µg), Cephalexin (Cl, 30 µg) Ciprofloxacin (CIP, 5 µg), Cefuroxime (CXM, 30 µg) followed by 70% to Erythromycin (E, 15 µg) Rifampin (RA, 5 µg), Ceftaxidime (CAZ, 30 µg) follow by 60%; Amoxicillin (AX, 25 µg), and 40% sensitivity to Penicillin (P, 10 µ) show in Table 5.

Table 5. *Antimicrobial susceptibility test for Staphylococcus aureus.*

Antibiotic discs	Inhibition zone			Total
	Sensitive	Resistant	Intermediates	
Ciprofloxacin	8	2	0	10
	80.00%	20.00%	0.00%	100.00%
Ceftriaxone	9	1	0	10
	90.00%	10.00%	0.00%	100.00%
Clindamycin	8	2	0	10
	80.00%	20.00%	0.00%	100.00%
Vancomycin	8	2	0	10
	80.00%	20.00%	0.00%	100.00%

Penicillin	4	6	0	10
	40.00%	60.00%	0.00%	100.00%
Amikacin	9	1	0	10
	90.00%	10.00%	0.00%	100.00%
Cephalexin	8	2	0	10
	80.00%	20.00%	0.00%	100.00%
Cefuroxime	8	2	0	10
	80.00%	20.00%	0.00%	100.00%
Erythromycin	7	3	0	10
	70.00%	30.00%	0.00%	100.00%
Amoxicillin	6	3	1	10
	60.00%	30.00%	10.00%	100.00%
Rifampin	7	3	0	10
	70.00%	30.00%	0.00%	100.00%
Ceftazidime	7	2	1	10
	70.00%	20.00%	10.00%	100.00%
Total	89	29	2	120
	74.20%	24.20%	1.70%	100.00%

6 from all isolates *Staphylococcus aureus* which were penicillin resistant were tested to determine if they are methicillin resistant *Staphylococcus aureus* by using methicillin (ME 10 µg) and Oxacillin (OX 1 µg) the result showed that 100% resistant to methicillin (ME 10 µg) and Oxacillin (OX 1 µg) as show in Table 6.

Table 6. *Antimicrobial susceptibility test for Staphylococcus aureus to detect MRSA.*

Antibiotic discs	Inhibition zone	Total
	Resistant	
Penicillin	6	6
	100.00%	100.00%
Oxacillin	6	6
	100.00%	100.00%
Methicillin	6	6
	100.00%	100.00%
Total	18	18
	100.00%	100.00%

Antimicrobial susceptibility test for Escherichia coli

One isolated *Escherichia coli* was tested using 10 antibiotics which showed 100%. Sensitive to gentamycin, cefotaxime, cefixime, erythromycin, ampicillin, imipenem, amikacin, ciprofloxacin, trimethoprim/sulfolmethoxazole, norfloxacin (Table 7).

Table 7. *Antimicrobial susceptibility test for Escherichia coli.*

Antibiotic discs	Inhibition zone		Total
	Sensitive	Resistant	
Gentamycin	1	0	1
	100.00%	0.00%	100.00%
Cefotaxime	1	0	1
	100.00%	0.00%	100.00%
Cefixime	1	0	1
	100.00%	0.00%	100.00%
Erythromycin	1	0	1
	100.00%	0.00%	100.00%
Ampicillin	0	1	1
	0.00%	100.00%	100.00%
Imipenem	1	0	1
	100.00%	0.00%	100.00%
Amikacin	1	0	1
	100.00%	0.00%	100.00%
Trimethoprim/sulfolmethoxazole	1	0	1
	100.00%	0.00%	100.00%
Ciprofloxacin	1	0	1
	100.00%	0.00%	100.00%
Norfloxacin	1	0	1
	100.00%	0.00%	100.00%
Total	9	1	10
	90.00%	10.00%	100.00%

Discussion

The present study showed that the most common etiologic agent isolated from patient with community acquired pneumonia was *Klebsiella pneumoniae* (42.8%). This result is consistent with other result by Liam [8] who showed that *Klebsiella pneumoniae* was the most frequently isolated pathogen and not compatible with Johansson et al. [9] who showed that *Streptococcus pneumoniae* was the most common isolated pathogen, also not compatible with Goel et al. [10] and Khawaja et al. [11] whom showed that *Pseudomonas aeruginosa*, *Staphylococcus aureus* was the most frequently isolated pathogen respectively this variation may be due to racial and geographical variations between each area. The most affected age group in this study were those of the age 16-26 (33.3%) and The present findings seem to differ from the results of Huijts et al. [12] which showed that the most affected age group was those above the age of 60 y with percentage of

55.8%. Torres et al. [13] reported that the community acquired pneumonia was statistically associated with alcoholic abuses, similar to our results which provided that significant relationship between occurring of community acquired pneumonia and alcoholic abuses. Previous studies done by Farr et al. [14] and other one by Torres et al. [13] showed increasing risk of CAP associated with smoking disagreement with the present result which show statistically insignificant relation between smoking and CAP (P-value=0.899). As shown in present study all isolated ESBL producer *Klebsiella pneumoniae* were 100% resistant to Cefepime (CPM), Ceftriaxone (CL), Ceftazidime (CAZ) and Cefotaxime (CTX), which is relatively high than that found by Romanus et al. [15] found that all ESBL producer *Klebsiella pneumoniae* were resistant to Ceftazidime 69%, Cefotaxime 74% and Ceftriaxone 79.6%. All isolated *Pseudomonas aeruginosa* was found to be 100% susceptible to Meropenem followed by 92.3% susceptible to Cefepim, Ceftazidime, Gentamicin, Aztreonam, and Imipenem and less susceptibility found to Piperacillin with percentage 53.8% with relatively similar to study done by Sadar et al. [16] who reported that the most effective antibiotics against *Pseudomonas aeruginosa* were Amikacin, Meropenem, Piperacillin/tazobactam, Imipenem and Aztreonam with 68.7%, 67.1%, 67.1%, 65.5 %, 42.1% susceptibility respectively. In the present study 6/10 (60%) *Staphylococcus aureus* were methicillin resistant this result relatively high to that found by Francis [17] who report that 4 patients were identified with severe community acquired methicillin resistant *Staphylococcus aureus* (MRSA) pneumonia. All isolate MRSA were found to be resistant to Penicillin, Methicillin, Oxacillin and susceptible to Ceftriaxone, Amikacin and Rifampin with 83.3% susceptibility reduce to 66.7% for Vancomycin, 50.0% Erythromycin and 33% for Amoxicillin, which is slightly similar to study done by Hageman [18] who reported that antimicrobial susceptibility test results for the isolated MRSA showed that all isolate were resistant to Oxacillin but susceptible to Rifampin and Vancomycin.

Conclusion

The study concluded that *Klebsiella pneumoniae* was the major etiological agents of community-acquired pneumonia among enrolled patients. The frequency of ESBL producer *Klebsiella pneumoniae* in patients with community acquired pneumonia is relatively high. Most of the isolated *Staphylococcus aureus* were found resistant to Methicillin and Oxacillin consider MRSA and all of the isolated *Pseudomonas aeruginosa* were found susceptible to Meropenem. Further studies are recommended for the detection of the other bacterial species in sputum samples coupled with viral detection using polymerase chain reaction and also Studies addressing the detection of anti-bigrams resistance gene of the bacteria causing community acquired pneumonia are also needed.

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